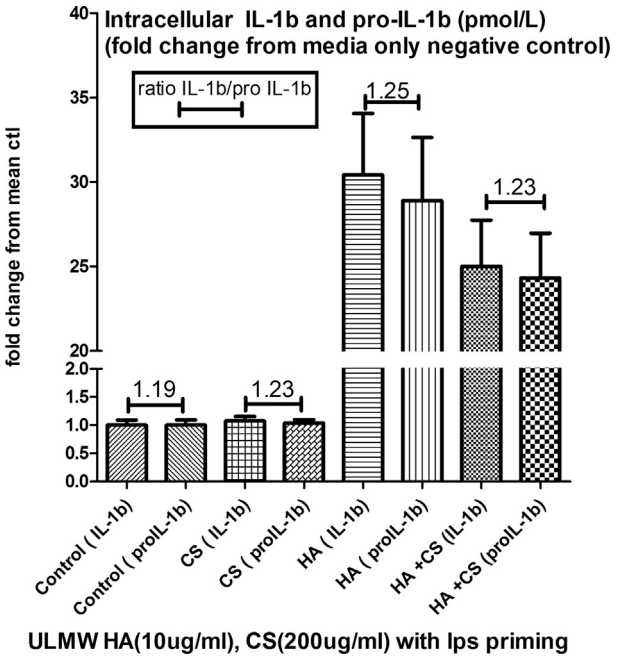

Conclusions: HA fragments (<289 kDa) induced an inflammatory response in THP-1 macrophages which could be attenuated by CS. An anti-inflammatory effect at the level of the inflammasome would be expected to decrease intracellular caspase-1 activity thereby decreasing the ratio of IL-1β to proIL-1β. Since we did not observe this, it can be concluded that the anti-inflammatory effect of CS is upstream of the inflammasome.

410
THE INFLAMMATORY PROFILE OF LUBRICIN

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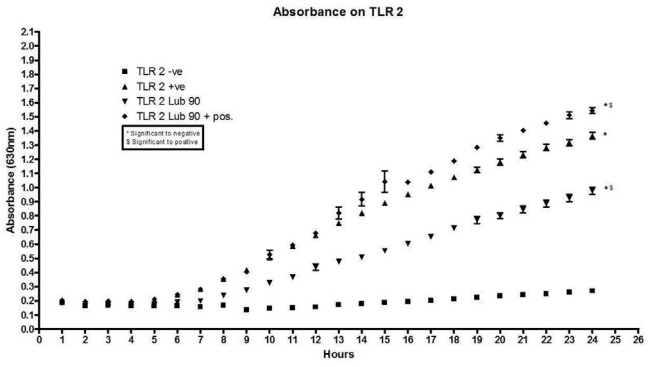
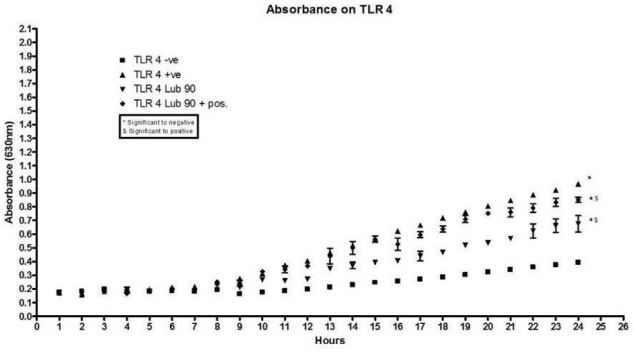
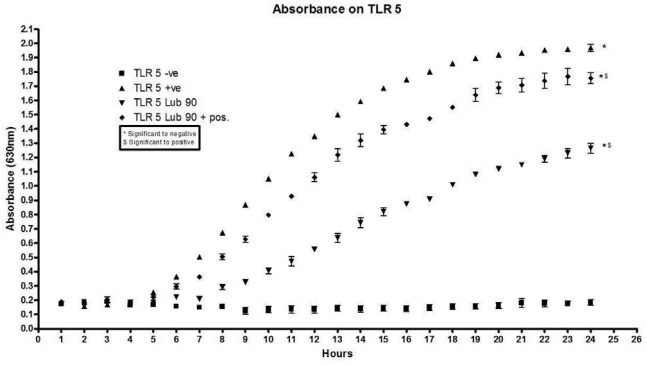
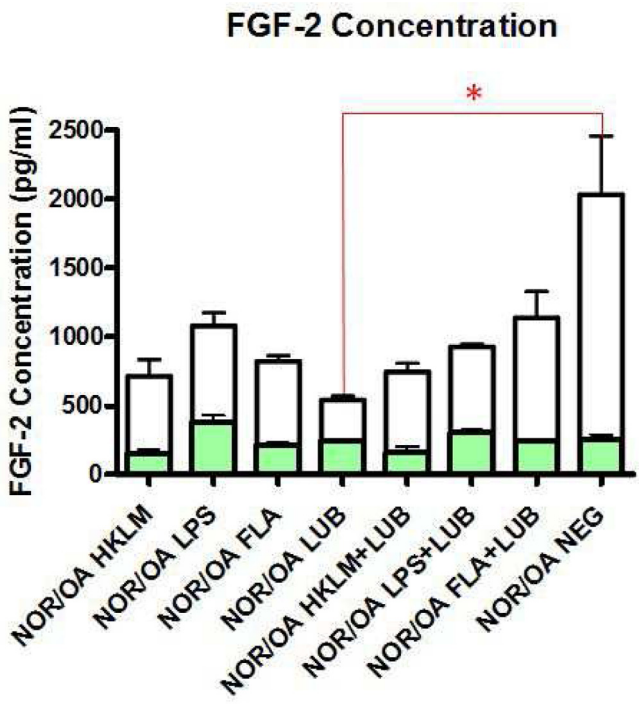
Purpose: Lubricin is a mucin-like glycoprotein, synthesized and secreted by cells that line the joint surface, that is critical for the normal lubricating mechanisms of articular cartilage, and its absence can lead to premature cartilage degeneration. Our initial screening results have demonstrated that lubricin can bind to and activate Toll-like receptors (TLRs), a class of receptor responsible for monitoring infection and immunity. The objective of this study was to validate this observation using genetically modified TLR overexpressing cell lines and test the effects of recombinant lubricin on the cytokine expression in Normal and Osteoarthritic (OA) human synovial fluid cells.
Methods: Three genetically modified human embryonic kidney (HEK) cell lines for TLRs 2, 4, and 5 were used in this experiment which produce Secreted Alkaline Phosphatase (SEAP) following TLR activation. The cells were exposed to the ligand and the absorbance (630nm) was measured after a 24 hour incubation period. These ligands were either the positive controls for the TLRs (Heat-killed Listeria Monocytogenes for TLR2, Lipopolysaccharides for TLR4, and Flagellin for TLR5), recombinant lubricin (90ug/ml) or hyaluronic acid (HA; MW 5, 20, and 132 kDa). The cell supernatant was collected after 24 hours for cytokine analysis using the Luminex multiplex platform. Normal and OA human synovial fluid cells were obtained from patient biopsies and purified following an immune cell depletion. They were then assayed for cytokines using Luminex following introduction of the same ligands.
Results: Lubricin activates TLR2, TLR4, and TLR5 receptors within 24 hours. For TLR2, lubricin in addition to the positive TLR control ligand elicits a greater response than the positive alone as observed by the absorbance. For the TLR4 and TLR5, lubricin with the respective positive controls elicits a lesser response than the positives alone.

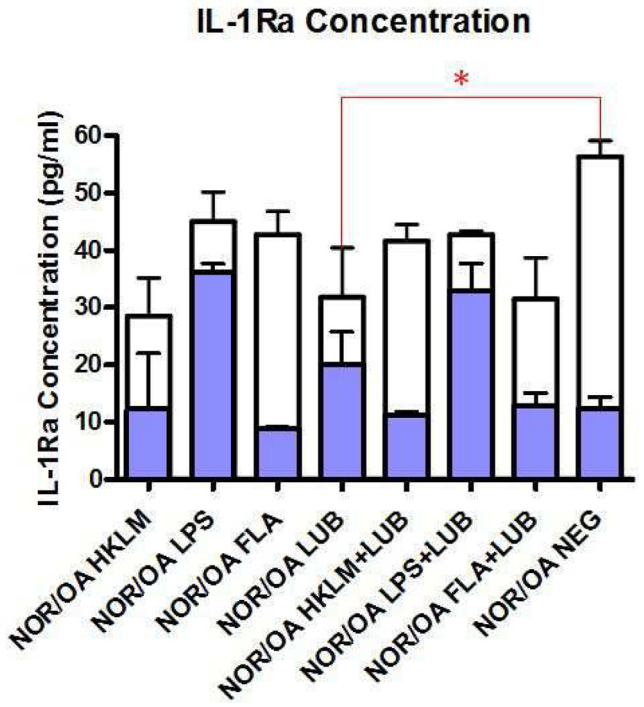






Luminex analysis revealed that the cytokines, GRO and IL-8, are expressed in the presence of the positive control for TLR 2, however, this effect is more pronounced when lubricin is introduced in conjunction with the positive control. The same effect is also observed for TLR4 and the IL-8 cytokine following lubricin + TLR 4 positive control introduction. TLR5, in the presence of its positive control is also shown to express GRO and IL-8, however, lubricin with the positive control results in lower concentrations than the positive control alone. The luminex analysis for the lubricin vs the lubricin + HA conditions did not reveal any significant differences regardless of fragment size of HA tested. Flow cytometry revealed the presence of TLR 2s and 5s in Normal and OA cells, with OA cells exhibiting greater numbers of both receptors. TLR 4s were present in limited amounts in both Normal and OA cells. Lubricin introduction to Normal cells does not elicit a response, however, OA cells are seen to down-regulate FGF-2 and IL-1Ra.
Interestingly, in OA cells, the cytokines G-CSF, IL-6, and IP-10 show a reduction in expression following lubricin introduction with lipopolysaccharides. This reduction is also seen in Normal cells, but only in the response of IL-6.
Conclusions: Lubricin can bind to and activate TLRs, and the effect is observed to differ based on the TLR type and whether or not it is present





with the respective positive controls. This effect is present in both the HEK and human synovial fluid cells.

411

STUDIES ON CORRELATION BETWEEN ANGIOGENESIS AND THE SYNOVIAL AQUAPORINS-1 EXPRESSION IN PATIENTS WITH KNEE OSTEOARTHRITIS

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Purpose: To explore the effect of the AQP-1 expression and angiogenesis on the KOA synovitis and to provide the experimental basis for further study of knee osteoarthritis by observing angiogenesis and the synovial aquaporins-1 expression in patients with knee osteoarthritis and analyzing their correlations.

Methods: according to the Kellgren – Lawrence (K/L) classification and the American rheumatism association KOA standards, 50 patients are divided into three groups: early KOA group, late KOA group and normal group. Then macroscopic observation of knee joint sample line and histological observation under a microscope are carried out, and synovial histological changes are evaluated by Krenn synovitis score. The AQP1 and CD34 expression in the synovial tissue are decided through the immunohistochemical detection. New endothelial cells marked by Microscope CD34 monoclonal antibody are counted, and the microvascular density (MVD) is calculated. With Pearson linear correlation analysis, the correlations between MVD, synovitis score,and the expression rate of AQP-1 positive cells are analyzed respectively.

Results: (1) Knee joint synovials in the early KOA group and late KOA group find inflammatory changes. The main changes are synovial lining layer hyperplasia, vascular proliferation and inflammatory cell invasion. The synovial inflammation of KOA group scores markedly higher than that of the normal group, with comparatively sharp difference ($p<0.05$), and the synovial inflammation of the early KOA group levels higher than other two groups, with rather sharp difference ($p<0.05$).(2)The immunohistochemical detection results show that compared with the normal synovial tissue, AQP-1 and MVD of the KOA group have a higher expression ($p<0.05$), and compared with the late KOA group, AQP-1 and MVD of the early KOA group find a higher expression ($p<0.05$).(3)The MVD value shows markedly positive correlation with the synovial inflammation score ($r=0.825, P<0.01$);the AQP-1 positive expression rate indicates significantly positive correlation with the synovial cell inflammatory score ($r=0.827, P<0.01$);the AQP-1 positive cell expression rate has obviously positive correlation with the MVD value ($r=0.894$, $P<0.01$).

Conclusions: (1) AQP-1 expression and new angiogenesis of the KOA group increase significantly compared with those of the normal group, and those of the early KOA group grow relatively compared with those of the late KOA group; and the higher AQP-1 expression may be associated with synovial angiogenesis. (2)Through coordinating the higher AQP-1 expression in the KOA synovium and angiogenesis may have the effect on the formation of the patients' KOA synovitis and the occurrence and development of KOA.

412

MEDIATION OF INFLAMMATION INDUCED EARLY PROTEASE ACTIVATION IN KNEE JOINT EXPLANTS THROUGH TIMELY INTERVENTION WITH GLUCOCORTICOIDS

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Purpose: Severe injuries to the knee joint, such as anterior cruciate ligament (ACL) tears and/or meniscal damage, often results in accelerated development of osteoarthritis (OA). It is hypothesized that interplay between altered mechanics and inflammation induced biological changes following knee joint injury, may be causative factors for OA development. Clinical evidence suggests that inflamed synovium and fat pad can add to adjacent articular cartilage damage. Following ACL rupture and/or surgery, the synovium and fat pad exhibit increased mRNA levels for inflammatory and degradative markers. We tested the hypothesis that early inhibition of inflammation was essential for suppressing the resulting upregulation of degradative proteinases. Methylprednisolone acetate (MPA; Depo-Medrol®, Pfizer) is a corticosteroid that is commonly clinically used for mitigating inflammation in many chronic inflammatory diseases. The present study evaluated the efficacy of using MPA for suppression of inflammation and consequently the degradative proteases in the synovium and fat pad tissue. Evaluation of the synovium and fat pad tissue responsiveness could provide relevant prognostic information, which may indicate that early intervention following severe knee injuries is essential to prevent initial cartilage degradation.

Methods: Hind limbs from normal 7-9 month old, female Suffolk Cross sheep were obtained following sacrifice and fat pad (6mm) and synovium tissue explants (6mm) were harvested from the knee joint. The explants were equilibrated in an incubator overnight in serum free Dulbecco's Modified Eagle's Medium (DMEM) at 37°C. The explants